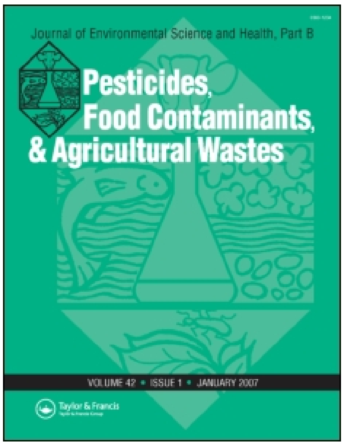

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Screening *Capsicum* Accessions for Capsaicinoids Content

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Ninety *Capsicum* accessions selected from the USDA *Capsicum* germplasm collection were screened for their capsaicinoids content using gas hromatography with nitrogen phosphorus detection (GC/NPD). Fresh fruits of *Capsicum chinense*, *C. frutescens, C. baccatum, C. annuum*, and *C. pubescens* were extracted with methanol and analyzed for capsaicin, dihydrocapsaicin, and nordihydrocapsaicin. Mass spectrometry of the fruit crude extracts indicated that the molecular ions at m/z 305, 307, and 293, which correspond to capsaicin, dihydrocapsaicin, and nordihydrocapsaicin, respectively, have a common benzyl cation fragment at m/z 137 that can be used for monitoring capsaicinoids in pepper fruit extracts. Capsaicin and dihydrocapsaicin were the dominant capsaicinoids detected. Capsaicin concentrations were typically greater than dihydrocapsaicin. Concentrations of total capsaicinoids varied from not detectable to 11.2 mg fruit^{-1}. Statistical analysis revealed that accession PI-441624 (*C. chinense*) had the highest capsaicin content (2.9 mg g^{-1} fresh fruit) and accession PI-497984 (*C. frutescens*) had the highest dihydrocapsaicin content (2.3 mg g^{-1} fresh fruit). Genebank accessions PI-439522 (*C. frutescens*) and PI-497984 contained the highest concentrations of total capsaicinoids.

Key Words: Hot peppers; Fruit extracts; Capsaicin; Dihydrocapsaicin; Mass spectra; Pungency.

INTRODUCTION

Environmentally compatible pest-control agents for use on vegetable crops are needed to replace pesticides that are ineffective, that have been withdrawn for regulatory reasons, or whose costs are prohibitive. The need for new control

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agents is also a result of the increasing difficulty of managing pesticide resistance. Botanical pesticides offer potential as substitutes, for, or supplements to be used with, synthetic pesticides,[1–3] particularly when two or more active components are combined to provide novel modes of action against a wide variety of pests. The likelihood of the targeted pests developing cross-resistance will be reduced as a result of the pest's difficulty in adapting simultaneously to a group of bioactive compounds. This could result in a need for fewer pesticide applications and result in a significant savings for organic growers and limited resource farmers.

The genus *Capsicum* (Family: Solanaceae) contains five commonly cultivated species (*C. annuum* L., *C. frutescens* L., *C. chinense* Jacq., *C. baccatum* L., and *C. pubescens* Ruiz & Pav.). Varieties of these, and other *Capsicum* spp., exhibit varying degrees of pungency that reflect the relative concentrations of capsaicin, dihydrocapsaicin, nordihydrocapsaicin (Fig. 1), and other analogs[4,5] that are known collectively as capsaicinoids.[6] Capsaicin [N-vanillyl-8-methyl-6-(E)noneamide] is the most pungent member in this group. Capsaicin and dihydrocapsaicin accounted for an estimated 80–95% of naturally occurring capsaicinoids in peppers.[7,8] Other forms are generally present in trace amounts. Scotch Bonnet and Habanero-type peppers are regarded as examples of extremely pungent forms of *Capsicum chinense*,[9] whereas Bell-type peppers are considered non-pungent forms of *C. annuum*. However, the concentrations of

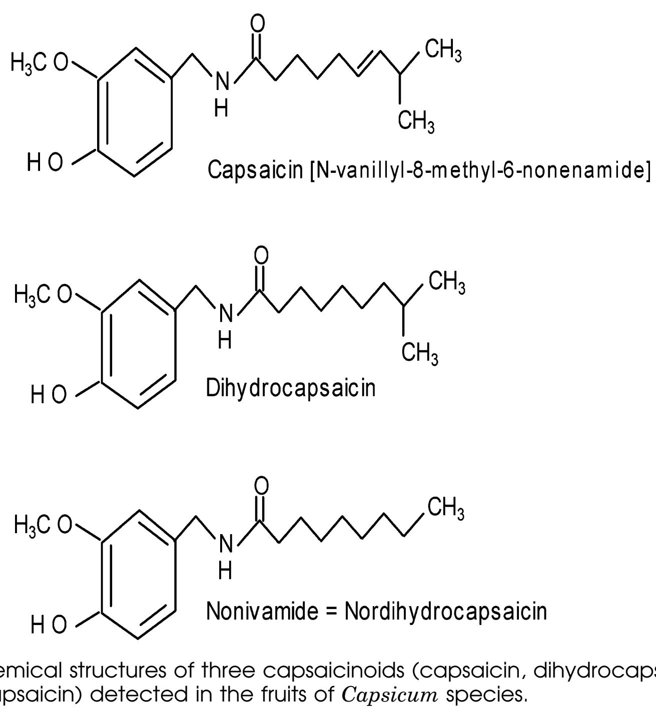

Figure 1: Chemical structures of three capsaicinoids (capsaicin, dihydrocapsaicin, and nordihydrocapsaicin) detected in the fruits of *Capsicum* species.

individual capsaicinoids and the proportion of capsaicin/dihydrocapsaicin fluctuate within and among species.[10] Absolute capsaicinoid concentrations are subject to a variety of environmental, cultural, and other factors.[11,12]

At the present time, 90% of U.S. chili pepper production occurs in New Mexico, eastern Arizona, and western Texas.[13] Pungent chili varieties are grown for their food value, health-promoting properties,[14] and also as a source of capsaicinoids that have variety of medicinal uses.[15] Capsaicin and dihydrocapsaicin exhibited considerable antioxygenic activity.[16] Studies carried out using mixtures of 64.5% capsaicin and 32.6% dihydrocapsaicin have indicated that capsaicinoids are not carcinogenic in mice.[17] In the absence of known toxicological concerns from the ingestion of capsaicin and other capsaicinoids, the EPA does not believe a tolerance for capsaicin is needed to protect the public health.[18]

The scientific literature suggests that extracts or powders from the fruit of pungent pepper varieties possess insecticidal activity. Hot pepper (*Capsicum* spp.) was superior to other plant extracts in protecting bean (*Phaseolus vulgaris*) plants from various insect pests including the foliar beetle, *Ootheca bennigseni*, and larvae of pod borers, *Maruca testulais* and *Heliothis armigera*. Hot pepper extracts were found as effective as lindane (a synthetic organochlorine insecticide) in protecting bean plants from insect pests.[19] Cowles, Keller, and Miller[20] reported that chili pepper powder deterred oviposition of the onion fly, *Delia antiqua*. Capsaicin in hot pepper has been reported to reduce larval growth of the spiny bollworm, *Earias insulana*.[21] The use of oleoresin from *Capsicum* has been reported effective as a repellent against cotton pests.[22] Capsaicin can provide better control of cabbage worms than Karate (λ-cyhalothrin), a synthetic insecticide.[23]

The USDA *Capsicum* germplasm collection contains many thousands of accessions of *Capsicum* spp.,[24] although only limited information is currently available on the capsaicinoid content of the fruit of these accessions. The objectives of the present investigation were (1) to quantify the major capsaicinoids (capsaicin and dihydrocapsaicin) in fruits of *Capsicum chinense*, *C. frutescens, C. baccatum, C. annuum*, and *C. pubescens* accessions obtained from the USDA *Capsicum* germplasm ollection, and (2) to screen and select candidate accessions from among those for mass production of capsaicinoids from hot pepper fruits for future use as alternative insecticides.

MATERIALS AND METHODS

Twenty plants of each accession were established in the greenhouse in the spring of 2004 and transplanted to the field in a sandy-loam soil (56.5% sand, 24.3% silt, and 19.2% clay) containing 1.3% organic matter at the Georgia

Experiment Station (Griffin, GA) in June. Accessions were selected to represent all five cultivated species, and a cross section of the geographic range of each of these. Fruits from 26 accessions of *Capsicum chinense*, 31 accessions of *C. frutescens*, 20 accessions of *C. baccatum*, 12 accessions of *C. annuum*, and 1 accession of *C. pubescens* were harvested at random from field-grown plants in the fall of 2004 and transported to Kentucky State University (Frankfort, KY) for capsaicinoid analysis.

Total capsaicinoids were extracted by blending 10 fresh fruit of comparable size in methanol for 1 min. The solvent extracts were decanted through 55 mm Whatman 934-AH glass microfiber filter discs (Fisher Scientific, Pittsburg, PA) and concentrated in a rotary vacuum evaporator (Buchi Rotovapor, Model 461, Flawil, Switzerland) at 35°C, chased with nitrogen gas (N_2), and reconstituted in 10 mL of methanol. Each extract was subsequently passed through a 0.45 μm GD/X disposable syringe filter (Fisher Scientific, Pittsburgh, PA). One μL of this filtrate was injected into a gas chromatograph (GC) equipped with a nitrogen-phosphorus detector (NPD). GC separations were accomplished using a 25 m $\times$ 0.20 mm ID capillary column with 0.33 μm film thickness (HP-1). Operating conditions were 230°C, 250°C, and 280°C for injector, oven, and detector, respectively, and the carrier gas (He) flow rate was 5.2 mL min^{-1}. Peak areas were determined using a Hewlett-Packard (HP) model 3396 series II integrator. Quantifications were based on average peak areas of 1 μL injections obtained from external standard solutions of capsaicinoids prepared in methanol. Under these conditions, retention times (R_t) were 9.06, 11.50, 11.75 min, for nordihydrocapsaicin, capsaicin, and dihydrocapsaicin, respectively. Peak identities were confirmed by consistent retention time and coelution with standards under the conditions described above. A HP gas chromatograph (GC) model 5890A equipped with a mass chromatograph operated in total ion monitoring (GC/MS) with electron impact ionization (EI) mode and 70 eV electron energy was also used for identification and confirmation of individual peaks. The instrument was auto-tuned with perfluorotributylamine (PFTBA) at m/z 69, 210, and 502. Purified standards of capsaicin (N-vanillyl-8-methyl-6-nonenamide) and dihydrocapsaicin were obtained from Sigma-Aldrich Inc. (Saint Louis, MO, USA) and used to prepare calibration curves. To determine the recovery of the extraction, cleanup, and quantification procedure, concentrations of capsaicin and dihydrocapsaicin in the range of 20–200 μg g^{-1} fresh fruit were added to 20 g of bell pepper (*C. annuum*) fruits. Recoveries of the added capsaicin and dihydrocapsaicin were 98% and 95%, respectively.

Linearity over the range of concentrations was determined using regression analysis. Concentrations of the two dominant capsaicinoids, capsaicin and dihydrocapsaicin, as well as total capsaicinoids (capsaicin plus dihydrocapsaicin) in *Capsicum* species were statistically analyzed using ANOVA procedure. Means were compared using Duncan's LSD test.

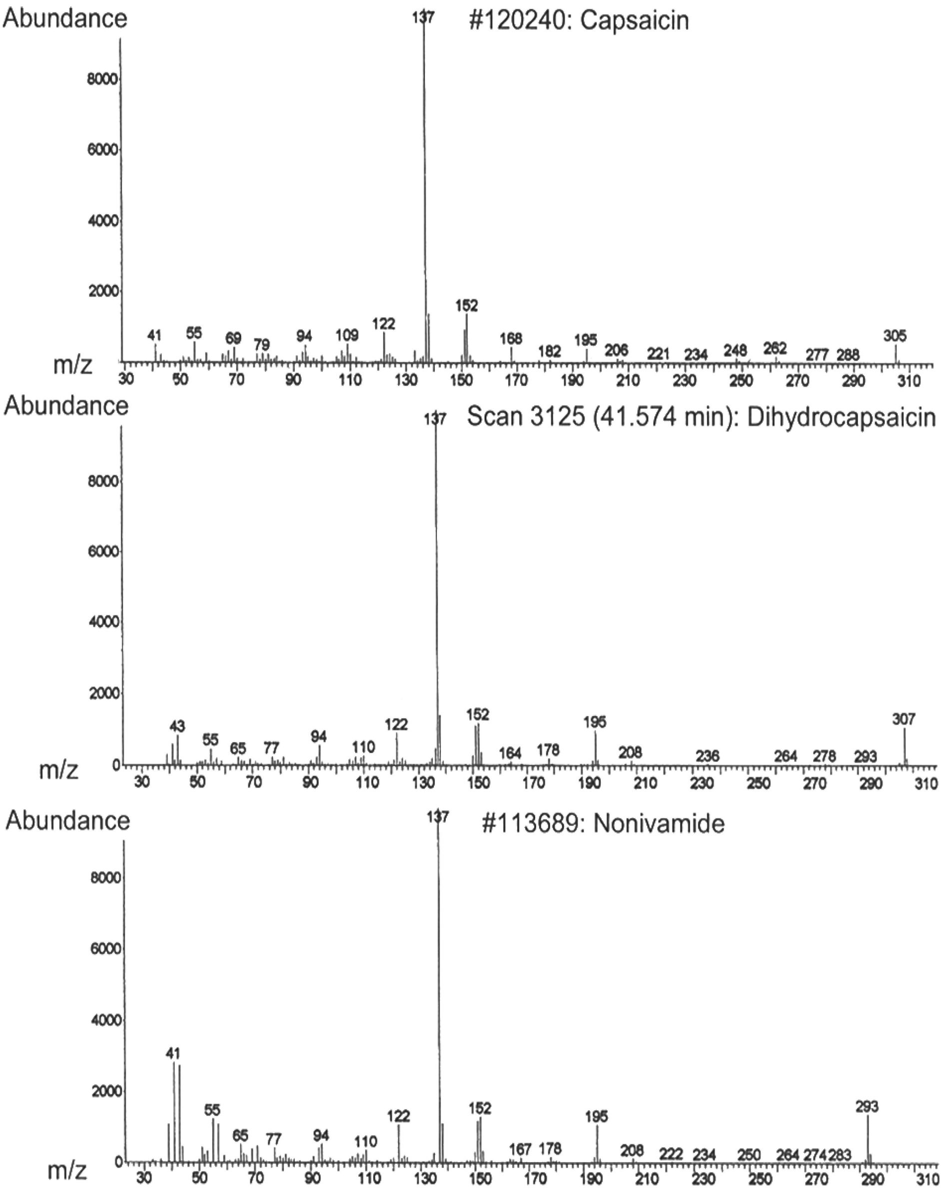

Figure 2: Electron impact mass spectrum of capsaicin ($C_{18}H_{27}NO_3$, upper), dihydrocapsaicin ($C_{18}H_{29}NO_3$, middle), and nonivamide known as nordihydrocapsaicin ($C_{17}H_{27}NO_3$, lower) detected in the fruits of *Capsicum* species indicating molecular ions of m/z 305, 307, and 293, respectively.

RESULTS AND DISCUSSION

Mass spectrometric analysis of fruit extracts revealed fragments with identical molecular ions at m/z 305, m/z 307, and m/z 293, in addition to other characteristic fragment ion peaks that were consistent with the assignment of the molecular formulae of capsaicin ($C_{18}H_{27}NO_3$), dihydrocapsaicin ($C_{18}H_{29}NO_3$), and nordihydrocapsaicin ($C_{17}H_{27}NO_3$), respectively. These had a common benzyl cation fragment ($C_8H_9O_2$, m/z 137) that was observed in all hot pepper extracts (Fig. 2). The retention time and mass spectra of capsaicinoids isolated from the fruits of *Capsicum* accessions matched those of their standards. Capsaicin and dihydrocapsaicin were the predominant capsaicinoids in the crude fruit extracts, although concentrations of each varied. Nordihydrocapsaicin was always present at very low concentrations when compared to capsaicin and

Table 1: Concentrations† of capsaicin and dihydrocapsaicin in the fruits of different accessions of *Capsicum chinense* grown under field conditions.

Accession	Capsaicin				Dihydrocapsaicin				Wt. (g) of each fruit‡
	$mg\ g^{-1}$	fruits	mg	$fruit^{-1}$	$mg\ g^{-1}$	fruits	mg	$fruit^{-1}$	
PI-224424	0.42	g	2.94	b	0.27	e	1.89	c	6.99
PI-224446	0.21	ijk	0.94	gh	0.12	jk	0.54	m	4.46
PI-257059	0.04	l	0.19	jk	0.00	o	0.00	o	4.73
PI-257063	0.27	hij	1.36	ef	0.29	e	1.46	def	5.02
PI-257065	0.32	h	1.31	ef	0.20	gh	0.82	ijk	4.09
PI-257104	0.22	hijk	1.28	efg	0.11	kl	0.64	klm	5.81
PI-257142	0.16	k	0.99	fgh	0.07	klm	0.43	mn	6.20
PI-290980	0.16	jk	0.74	hi	0.07	lm	0.32	n	4.61
PI-360723	0.32	h	1.64	de	0.16	ij	0.82	ijkl	5.14
PI-387833	0.72	f	1.44	de	0.38	d	0.76	jkl	2.00
PI-387836	0.31	hi	1.66	de	0.17	hi	0.91	hij	5.34
PI-438622	0.90	e	7.69	a	0.42	d	3.59	a	8.54
PI-439428	1.56	b	1.28	efg	1.15	a	0.94	hij	0.82
PI-441624	2.89	a	0.95	fgh	0.00	o	0.00	o	0.33
PI-560943	0.06	l	1.00	fgh	0.09	klm	1.51	def	16.74
PI-585253	0.23	hijk	2.21	c	0.11	kl	1.06	hi	9.61
PI-593925	1.54	b	3.22	b	0.72	c	1.50	de	2.09
GRIF-9117	0.00	l	0.00	k	0.00	o	0.00	o	5.47
GRIF-9271	0.31	hi	1.35	ef	0.25	ef	1.09	gh	4.36
GRIF-9272	0.48	g	2.88	b	0.22	fg	1.32	ef	6.00
GRIF-9273	1.33	cd	3.06	b	0.84	b	1.93	c	2.30
GRIF-9300	0.25	hijk	0.81	hi	0.25	ef	0.81	ijkl	3.23
GRIF-9317	0.72	f	2.50	c	0.7	c	2.43	b	3.47
GRIF-9320	0.06	l	0.76	hi	0.02	no	0.25	n	12.65
GRIF-9367	0.02	l	0.24	jk	0.05	mn	0.60	lm	11.90
GRIF-9368	0.43	g	1.76	d	0.40	d	1.64	d	4.09

† Detectability limits (minimum detectable concentration in μg divided by sample weight in g) for capsaicin and dihydrocapsaicin were similar and averaged 0.001 $\mu g\ g^{-1}$ fresh fruits.
‡ Average weight of each fresh pepper fruit ($n = 10$). Values within a column for each compound having different letter(s) are significantly different ($P < 0.05$) from each other, using Duncan's LSD test (SAS Institute).

Table 2: Concentrations† of capsaicin and dihydrocapsaicin in the fruits of different accessions of *Capsicum frutescens* grown under field conditions.

Accession	Capsaicin				Dihydrocapsaicin				Wt. (g) of each fruit‡
	mg g^{-1}	fruits	mg	fruit^{-1}	mg g^{-1}	fruits	mg	fruit^{-1}	
PI-159261	0.12	n	1.50	c	0.12	no	1.50	a	12.50
PI-194260	1.58	ef	0.96	ghi	1.29	d	0.79	defg	0.61
PI-197406	0.34	m	0.52	opq	0.09	o	0.14	mn	1.53
PI-209109	0.73	jk	1.25	def	0.57	l	0.97	cde	1.71
PI-224416	1.77	c	1.77	b	1.43	c	1.43	a	1.00
PI-224431	1.06	h	0.73	jklmn	0.89	ij	0.61	ghij	0.69
PI-238057	1.74	cd	1.32	cde	1.45	c	1.10	bc	0.76
PI-257051	1.46	f	0.88	hij	0.87	ij	0.52	jk	0.60
PI-257067	0.09	n	0.50	pq	0.00	o	0.00	n	5.56
PI-257077	2.01	b	0.70	jklmno	1.14	ef	0.40	jkl	0.35
PI-257083	0.40	m	0.64	klmnop	0.23	mn	0.37	kl	1.61
PI-257121	1.31	g	0.62	lmnopq	0.54	l	0.25	lm	0.47
PI-281419	1.53	ef	0.44	q	0.54	l	0.16	mn	0.29
PI-322717	1.63	de	0.78	ijklm	0.80	jk	0.38	kl	0.48
PI-358968	1.78	c	0.62	lmnopq	1.34	cd	0.47	jk	0.35
PI-387834	0.10	n	0.61	mnopq	0.09	o	0.55	hijk	6.13
PI-439234	0.56	l	0.97	gh	0.56	l	0.97	cde	1.73
PI-439521	0.92	i	0.81	hijkl	0.56	l	0.49	jk	0.88
PI-439522	2.39	a	1.70	b	1.71	b	1.21	b	0.71
PI-441648	1.46	f	1.40	cd	1.11	fg	1.07	bc	0.96
PI-487623	0.05	n	0.26	r	0.10	o	0.52	jk	5.15
PI-497984	1.80	c	0.45	pq	2.25	a	0.57	ijk	0.25
PI-555644	1.15	h	1.74	b	0.93	hi	1.40	a	1.51
PI-585257	1.27	g	0.55	nopq	1.25	de	0.54	jk	0.43
PI-593614	2.07	b	0.81	hijk	1.14	ef	0.44	jkl	0.39
PI-631137	1.06	h	0.66	klmnop	0.84	ijk	0.52	jk	0.62
PI-631139	1.58	ef	0.85	hij	1.45	c	0.78	efgh	0.54
GRIF-9228	0.84	ij	1.12	fg	0.75	k	1.00	cd	1.33
GRIF-9310	0.62	kl	1.36	cde	0.28	7m	0.61	fghij	2.19
GRIF-9319	1.59	ef	1.21	ef	1.02	gh	0.78	efghi	0.76
GRIF-9324	0.16	n	1.98	a	0.09	o	1.12	bc	12.39

† Detectability limits (minimum detectable concentration in μg divided by sample weight in g) for capsaicin and dihydrocapsaicin were similar and averaged 0.001 μg g^{-1} fresh fruits.
‡ Average weight of each fresh pepper fruit ($n = 10$). Values within a column for each compound having different letter(s) are significantly different ($P < 0.05$) from each other, using Duncan's LSD test (SAS Institute).

dihydrocapsaicin. Concentrations of nordihydrocapsaicin in fruits of *C. chinense*, *C. frutescens, C. baccatum, C. annuum*, and *C. pubescens* accessions averaged 0.75, 1.40, 0.81, 0.35, and 0.56 μg g^{-1} fresh fruit, respectively. Because of these low concentrations, no further efforts were made to quantify nordihydrocapsaicin in the fruit extracts.

Analysis of capsaicin and dihydrocapsaicin in *C. chinense* (Table 1), *C. frutescens* (Table 2), *C. baccatum* (Table 3), and *C. annuum* (Table 4) indicated that the concentrations and relative proportions of these capsaicinoids varied between and within species, as reported earlier.[10,25] C*apsicum chinense* accession PI-441624 had the highest concentration of capsaicin (2.9 mg g^{-1}

Table 3: Concentrations† of capsaicin and dihydrocapsaicin in the fruits of different accessions of *Capsicum baccatum* grown under field conditions.

	Capsaicin				Dihydrocapsaicin				
Accession	mg g^{-1}	fruits	mg	$fruit^{-1}$	mg g^{-1}	fruits	mg	$fruit^{-1}$	Wt. (g) of each fruit‡
PI-281408	0.23	ij	1.22	ghi	0.10	i	0.53	hi	5.32
PI-315020	0.26	hij	1.80	de	0.15	h	1.04	ef	6.92
PI-424732	0.08	klm	0.860	ijk	0.00	j	0.00	k	10.74
PI-497985	1.90	b	4.94	b	0.00	j	0.00	k	2.60
PI-543178	0.50	ef	1.20	ghi	0.31	f	0.74	gh	2.40
PI-560935	0.59	de	1.60	ef	0.37	e	1.00	ef	2.71
PI-585242	0.35	gh	1.33	fgh	0.21	g	0.80	fg	3.79
PI-590506	0.48	f	1.10	hi	0.45	d	1.04	ef	2.30
PI-596056	0.17	jkl	1.68	ef	0.11	i	1.09	e	9.90
PI-596057	0.07	lm	0.58	jkl	0.00	j	0.00	k	8.24
PI-633751	2.07	a	0.93	ij	1.09	b	0.49	hi	0.45
PI-633754	0.71	c	1.51	efg	0.29	f	0.62	ghi	2.13
PI-633755	0.44	fg	1.02	hi	0.18	h	0.42	ij	2.32
PI-633756	0.02	m	0.40	l	0.03	j	0.59	ghi	19.75
PI-633757	0.47	f	0.44	l	0.22	g	0.20	jk	0.93
PI-633758	0.35	gh	1.35	fgh	1.57	a	6.06	a	3.86
GRIF-9212	0.12	kl	2.10	cd	0.10	i	1.75	d	17.50
GRIF-9213	0.18	jk	2.17	c	0.17	h	2.05	c	12.08
GRIF-9217	0.30	hi	5.94	a	0.28	f	5.54	b	19.80
GRIF-9354	0.62	cd	0.55	kl	0.73	c	0.65	ghi	0.89

† Detectability limits (minimum detectable concentration in μg divided by sample weight in g) for capsaicin and dihydrocapsaicin were similar and averaged 0.001 μg g^{-1} fresh fruits.
‡ Average weight of each fresh pepper fruit ($n = 10$). Values within a column for each compound having different letter(s) are significantly different ($P < 0.05$) from each other, using Duncan's LSD test (SAS Institute).

Table 4: Concentrations† of capsaicin and dihydrocapsaicin in the fruits of different accessions of *Capsicum annuum* grown under field conditions.

	Capsaicin				Dihydrocapsaicin				
Accession	mg g^{-1}	fruits	mg	$fruit^{-1}$	mg g^{-1}	fruits	mg	$fruit^{-1}$	Wt. (g) of each fruit‡
PI-159264	0.34	d	2.79	a	0.00	d	0.00	g	8.22
PI-195299	0.91	b	2.07	b	0.61	a	1.38	bc	2.27
PI-414729	0.03	gh	0.34	f	0.05	cd	0.56	efg	11.24
PI-419133	0.07	fgh	0.77	e	0.08	cd	0.88	cde	11.01
PI-593598	1.12	a	0.67	e	0.32	bc	0.19	fg	0.60
GRIF-14486	0.16	ef	1.31	d	0.15	cd	1.23	bcd	8.18
GRIF-14487	0.18	e	0.62	e	0.25	bcd	0.86	cde	3.42
GRIF-14513	0.01	h	0.38	f	0.02	d	0.75	ef	37.64
GRIF-9149	0.10	efgh	2.11	b	0.10	cd	2.11	a	21.06
GRIF-9169	0.00	h	0.00	g	0.05	cd	1.04	cde	20.79
GRIF-9270	0.12	efg	1.75	c	0.12	cd	1.75	ab	14.61
GRIF-9277	0.56	c	1.51	d	0.45	ab	1.22	bcd	2.70

† Detectability limits (minimum detectable concentration in μg divided by sample weight in g) for capsaicin and dihydrocapsaicin were similar and averaged 0.001 μg g^{-1} fresh fruits.
‡ Average weight of each fresh pepper fruit ($n = 10$). Values within a column for each compound having different letter(s) are significantly different ($P < 0.05$) from each other, using Duncan's LSD test (SAS Institute).

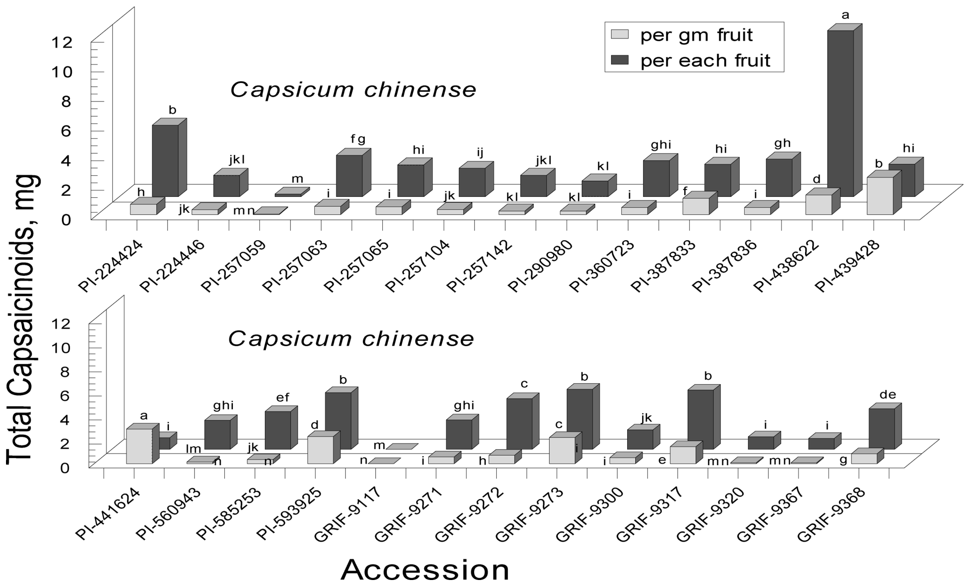

Figure 3: Concentrations of total capsaicinoids (capsaicin and dihydrocapsaicin) in the fruits of *Capsicum chinensi.* Bars accompanied by different letter(s) indicate significant differences ($P < 0.05$) using Duncan's LSD test.

fruit), while fruit extract of PI-439428 had the highest concentration of dihydrocapsaicin when compared to other accessions of that species (Table 1). Capsaicinoids were not detected at a level of 0.001 mg g^{-1} fruit in *C. chinense* accession Grif-9117. *Capsicum frutescens* accession PI-439522 had the highest concentration of capsaicin (2.4 mg g^{-1} fruit), while PI-497984 (*C. frutescens*) had the highest concentration of dihydrocapsaicin (2.3 mg g^{-1} fruit) (Table 2) in that species. *Capsicum baccatum* PI-633751 had the highest concentration of capsaicin (2.1 mg g^{-1} fruit) in the fruit extracts of this species, while PI-633758 (*C. baccatum*) had the highest concentration of dihydrocapsaicin (1.6 mg g^{-1} fruit). No dihydrocapsaicin was detected in *C. baccatum* PI-424732 (Table 3). *Capsicum annuum* PI-593598 had the highest concentration of capsaicin (1.1 mg g^{-1} fruit) among the *C. annuum* accessions examined, while PI-195299 had the highest concentration of dihydrocapsaicin (0.61 mg g^{-1} fruit). Dihydrocapsaicin was not detected in *C. annuum* PI-159264 at the detectability level of 0.001 mg g^{-1} fruit (Table 4). Only one accession (PI-387838) of *Capsicum pubescens* was analyzed in this study. The total capsaicinoids in PI-387838 averaged 0.7 mg g^{-1} fruit (1.92 mg capsaicinoids per fruit). In this accession, concentrations of capsaicin (0.37 mg g^{-1} fresh fruit) and dihydrocapsaicin (0.34 mg^{-1} fresh fruit) were not significantly different.

Capsaicinoid concentrations varied between accessions of the same species (Figure 3). In most cases, capsaicin concentrations were higher than

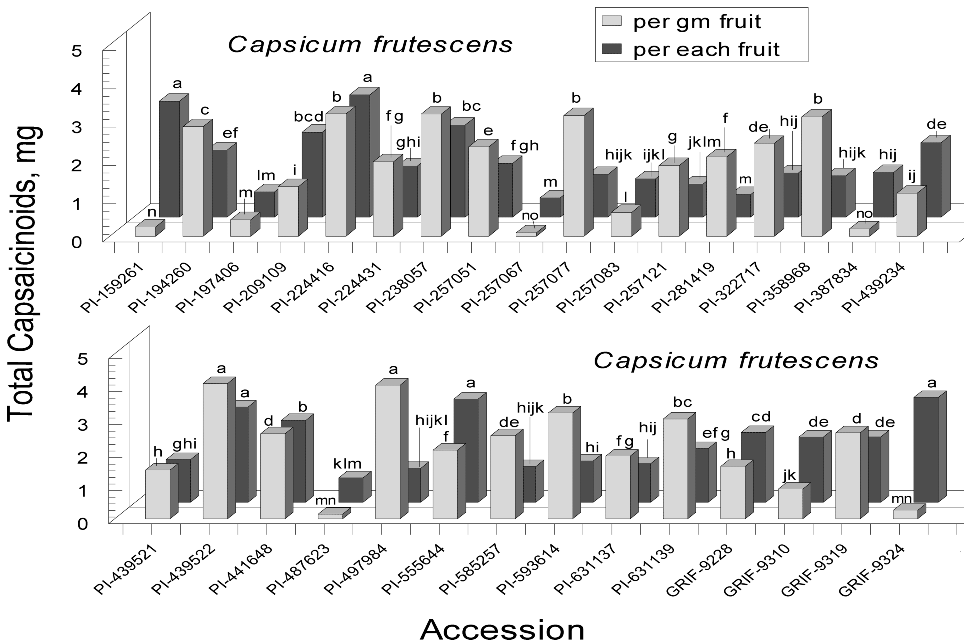

Figure 4: Concentrations of total capsaicinoids (capsaicin and dihydrocapsaicin) in the fruits of *Capsicum frutescens*. Bars accompanied by different letter(s) indicate significant differences ($P < 0.05$) using Duncan's LSD test.

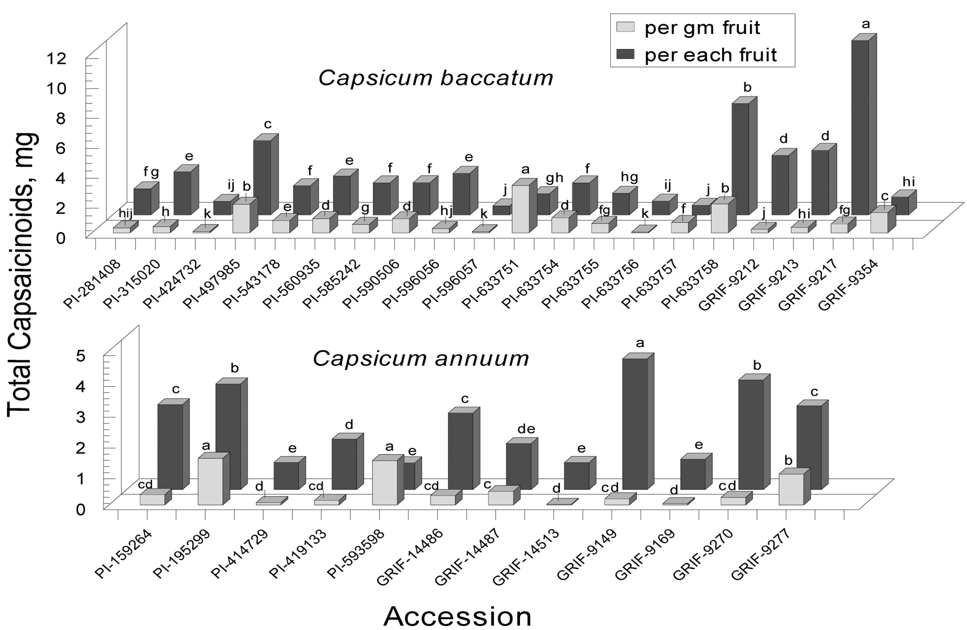

Figure 5: Concentrations of total capsaicinoids (capsaicin and dihydrocapsaicin) in the fruits of *Capsicum baccatum* (upper) and *Capsicum annuum* (lower). Bars accompanied by different letter(s) indicate significant differences ($P < 0.05$) using Duncan's LSD test.

dihydrocapsaicin, and total capsaicinoid content (capsaicin plus dihydrocapsaicin) varied from not detectable to 11.2 mg fruit^{-1}. Figures 3–5 illustrate the variability for total capsaicinoid concentrations among the accessions included in this study. Statistical analysis revealed that PI-441624 (Table 1) had the highest capsaicin content (2.9 mg g^{-1} fresh fruit), while PI-497984 (Table 2) had the highest concentration of dihydrocapsaicin (2.3 mg g^{-1} fresh fruit). Accession numbers PI-439522 and PI-497984 contained the highest concentrations of total capsaicinoids per fruit (4.09 and 4.05 mg g^{-1}, respectively) (Figure 4). In addition, PI-438622 (*C. chinensi*) contained the highest concentration of total capsaicinoids per fruit (11.2 mg fruit^{-1}) among all accessions analyzed (Figure 3).

CONCLUSIONS

Capsaicin is approved by the FDA for human use[26] and is currently registered for use as an animal repellent against birds, deer, rabbits, and squirrels.[27] Quantification of capsaicinoids in the selected accessions allowed us to identify genotypes with high levels of total capsaicinoids and enabled the prediction of the amount of each component that can be obtained per kilogram and per acre of hot peppers produced. Genebank accessions PI-441624, PI-497984, and PI-439522 were identified as potential candidates for the mass production of capsaicinoids, or for the breeding of varieties enhanced for capsaicin content. Future research objectives will include the development of novel formulations of capsaicinoids derived from the accessions characterized in this study, the monitoring of the effectiveness of the insecticidal activity of capsaicinoids, and persistence of capsaicinoids under field conditions.

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